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In Vitro and In Vivo Activities of Rifampin, Streptomycin, Amikacin, Moxifloxacin, R207910, Linezolid, and PA-824 against *Mycobacterium ulcerans*

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Seven antimicrobials were tested in vitro against 29 clinical isolates of *Mycobacterium ulcerans*. R207910 demonstrated the lowest MIC_{50} and MIC_{90}, followed by moxifloxacin (MXF), streptomycin (STR), rifampin (RIF), amikacin (AMK), linezolid (LZD), and PA-824. All but PA-824 demonstrated an MIC_{90} significantly less than the clinically achievable peak serum level. Administered as monotherapy to mice, RIF, STR, AMK, MXF, R207910, and LZD demonstrated some degree of bactericidal activity, whereas PA-824 failed to prevent mortality and to reduce the mean number of CFU in the footpads. Because 4 or 8 weeks of treatment by the combinations RIF-MXF, RIF-R207910, and RIF-LZD displayed bactericidal effects similar to those of RIF-STR and RIF-AMK, these three combinations might be considered as orally administered combined regimens for treatment of Buruli ulcer. Taking into account the cost, potential toxicity, and availability, the combination RIF-MXF appears more feasible for application in the field; additional experiments with mice are warranted to define further its activity against *M. ulcerans*. In addition, a pilot clinical trial is proposed to test the efficacy of RIF-MXF for treatment of Buruli ulcer.

Buruli ulcer, a major emerging disease that involves skin and soft tissues and may cause permanent disability (17), has been reported from more than 30 countries, mostly tropical, and has become the third most common mycobacterial disease in humans after tuberculosis and leprosy. Since 1980, the incidence of Buruli ulcer has increased dramatically in western African countries, such as Benin, Côte d'Ivoire, and Ghana (17). Approximately half of the victims are children aged 5 to 15 years; in some districts of these countries where Buruli ulcer is endemic, the detection rate of Buruli ulcer is greater than that of tuberculosis or leprosy (unpublished data). The etiologic agent is *Mycobacterium ulcerans*, a slow-growing mycobacterium that can be cultured on mycobacterial medium at 30 to 32°C. A unique characteristic of *M. ulcerans* is that it produces a family of toxic molecules, the mycolactones, which are responsible for the tissue destruction and local immunosuppression observed with Buruli ulcer lesions (25).

Until recently, chemotherapy has been considered ineffective (24); it has been postulated, without evidence, that antimicrobial agents fail to penetrate the lesions of Buruli ulcer because of the extensive necrosis (11, 21). Consequently, standard treatment has been wide surgical excision followed by skin grafting (24). However, surgical treatment is often not available in rural Africa, nor can most patients afford it; in addition, the disease has been reported to recur after surgery in 18 to 47% of cases (17). Therefore, development of an effective drug treatment is a research priority for controlling Buruli ulcer.

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After the demonstration that the combinations rifampin (RIF)-streptomycin (STR) and RIF-amikacin (AMK) displayed bactericidal activity against *M. ulcerans* in mice (3, 10, 19), a randomized clinical trial was launched in Ghana to assess the therapeutic effectiveness of RIF-STR for treatment of preulcerative (nodules and plaques) Buruli ulcer in humans. The results of this trial demonstrated that, after treatment for at least 4 weeks, *M. ulcerans* could no longer be cultured from the lesions (11). The therapeutic efficacy of RIF-STR was subsequently confirmed in an open trial in Benin (6). Of 224 patients who had been treated for 4 to 8 weeks with RIF-STR, the lesions of 211 (94.2%) patients were healed; 112 of the patients, most of whom exhibited larger ulcers, had received chemotherapy plus surgical excision, but the lesions of the remaining 99 patients, mostly ulcers of small to moderate size, were cured by chemotherapy alone. Of the 124 patients who were monitored for at least 12 months after their lesions had healed, local recurrence of the lesions was observed in only 3 (2.4%) (6). These data represent the first evidence that Buruli ulcer may be cured by antimicrobial agents without surgery (6). To promote the treatment of Buruli ulcer with RIF-STR in the field, a provisional guidance (27) has been prepared by the World Health Organization and is being circulated.

However, in rural Africa, ambulatory treatment that requires daily intramuscular injection of STR for 4 to 8 weeks is too demanding operationally and also carries the risk of transmission of human immunodeficiency virus infection. Development of an effective, orally administered combined regimen would greatly simplify the treatment of Buruli ulcer under field conditions. Therefore, there is an urgent need to identify antimicrobial agents that are highly active against *M. ulcerans* and can be administered orally.

Because *M. ulcerans* is a slow-growing mycobacterium, we

TABLE 1. Mean number (log_{10}) of CFU per footpad in various groups of mice

Regimen	Result at:							
			4 wk			8 wk		
	Day 0 (mean [±SD] CFU per footpad for the group)[a]	2 wk (mean [±SD] CFU per footpad for the group)	No. of mice culture positive/ total no. of mice	Mean (±SD) CFU per culture-positive footpad	Mean (±SD) CFU per footpad for the group[f]	No. of mice culture positive/ total no. of mice	Mean (±SD) CFU per culture-positive footpad	Mean (±SD) CFU per footpad for the group[f]
Untreated control[b]	6.35 ± 0.34	7.06 ± 0.26	13/13	6.83 ± 0.36	6.83 ± 0.36			
RIF alone		5.79 ± 0.26	3/10[c]	1.16 ± 0.58	<0.92 ± 0.32	0/9[d]		All 9 pads neg.
STR alone		4.57 ± 0.58	2/9[c]	1.45 ± 0.89	<0.96 ± 0.42	2/10[d]	0.30 ± 0	0.06 ± 0.13
AMK alone		4.09 ± 0.83	6/10[c]	1.66 ± 0.77	<1.33 ± 0.72	2/9[d]	1.14 ± 0.62	0.25 ± 0.55
MXF alone			10/10	4.09 ± 1.09	4.09 ± 1.09	8/10[e]	2.02 ± 1.10	<1.78 ± 1.09
R207910 alone			8/10	4.17 ± 0.89	3.90 ± 0.97	3/10[e]	2.97 ± 0.91	<1.00 ± 1.43
LZD alone			10/10	3.54 ± 1.89	3.54 ± 1.89	7/10[e]	2.35 ± 1.78	<1.69 ± 1.80
PA-824 alone			14/14	6.25 ± 0.20	6.25 ± 0.20			
RIF-STR		4.02 ± 0.62	0/9[d]		All 9 pads neg.	0/10[d]		All 10 pads neg.
RIF-AMK		3.27 ± 0.57	0/10[d]		All 10 pads neg.	0/10[d]		All 10 pads neg.
RIF-MXF			3/10[c]	0.82 ± 0	<0.82	0/10[e]		All 10 pads neg.
RIF-R207910			1/10[c]	2.46	<0.99 ± 0.52	1/10[e]	0.46	<0.19 ± 0.10
RIF-LZD			1/10[c]	0.82	<0.82	0/10[e]		All 10 pads neg.

[a] Treatment was begun 6 weeks after inoculation with 2.3×10^3 CFU of *M. ulcerans* per footpad, at which time inflammatory swelling with a lesion index (10) of 2 or 3 was observed for all inoculated footpads.
[b] After randomization, 35 mice were allocated to the control group: 10 were sacrificed at 2 weeks, 6 died during the fourth week of treatment, and all 19 surviving controls had developed severe inflammatory swelling footpads (lesion index of 4) and were sacrificed at 4 weeks. Unfortunately, during enumeration of CFUs, 6 of the 19 footpads at 4 weeks were contaminated; this was relatively common in the case of footpads that had developed a high lesion index.
[c] Undiluted and 1:10-diluted tissue suspensions of an inoculated footpad were plated, in triplicate, on Löwenstein-Jensen medium.
[d] The entire volume (2 ml) of the undiluted tissue suspension from each footpad was plated on 10 tubes of Löwenstein-Jensen medium.
[e] In addition to the 1:10-diluted tissue suspension, which was plated in triplicate on Löwenstein-Jensen medium, 1.5 ml of the undiluted tissue suspension from each footpad was plated on seven tubes of the same medium.
[f] neg., negative.

focused our screening on orally administered, newer antimicrobial agents that had displayed promising activity against *M. tuberculosis* or *M. leprae*, also slow-growing mycobacteria. Recent studies have demonstrated that both moxifloxacin (MXF), a newer fluoroquinolone, and R207910, a diarylquinoline, exhibit powerful bactericidal activity against *M. tuberculosis* (2, 14) and *M. leprae* (7, 15) in mice; that PA-824, a nitroimidazopyran, exhibits excellent activity both in vitro and in vivo against *M. tuberculosis* (18, 22, 23) and modest activity in vivo against *M. leprae* (15); and that linezolid (LZD), an oxazolidinone, is active against *M. tuberculosis* (1, 8), most rapidly growing mycobacteria (26), and slowly growing nontuberculous mycobacteria (5) and also shows modest activity against *M. leprae* in mice (15). Therefore, we measured the activities of these four agents against *M. ulcerans* both in vitro and in vivo and compared them to RIF, STR, and AMK as positive controls.

MATERIALS AND METHODS

Antimicrobial agents. R207910 was provided by Johnson & Johnson (Beerse, Belgium) and PA-824 by the Global Alliance for TB Drug Development (New York, N.Y.). MXF was purchased from Bayer Pharma (Puteaux, France), LZD from Pfizer (Paris, France), RIF from Gruppo Lepetit (Anagni, Italy), STR from Panpharma (Fougeres, France), and AMK from Bristol-Myers Squibb (Paris, France).

***M. ulcerans* isolates.** The MICs of the seven antimicrobials against 29 clinical isolates of *M. ulcerans*, including the reference strain of *M. ulcerans* (ATCC 19423), were determined; all of the 29 isolates, except for isolate CU001, were kindly provided by F. Portaels, Institute of Tropical Medicine, Antwerp, Belgium. Among the 29 isolates, 8 had been isolated from patients in Benin, 5 from Australia, 3 from Democratic Republic of the Congo, 2 each from Mexico and Côte d'Ivoire, and 1 each from Ghana, Togo, China, Malaysia, Cameroon, French Guiana, Angola, Suriname, and Papua New Guinea. All of the isolates had been regularly subcultured through Löwenstein-Jensen medium. Isolate CU001, which we isolated from a patient in Côte d'Ivoire, was the only isolate employed for testing the in vivo activities of antimicrobial agents against *M. ulcerans* (3, 9, 10).

Determination of the MICs against *M. ulcerans*. Fresh colonies of *M. ulcerans* were collected from the Löwenstein-Jensen medium and suspended in distilled water; the turbidity of the resulting suspensions was then adjusted with distilled water to match that of a standard 1-mg/ml suspension of *M. bovis* BCG (containing approximately 10^8 CFU per ml), after which the suspensions were further diluted to 10^{-1} and 10^{-2} mg/ml. The MICs were determined on 10% oleic acid-albumin-dextrose-catalase-enriched 7H11 agar medium. Both RIF and R207910 were dissolved in dimethylformamide (DMF); MXF, LZD, STR, and AMK were dissolved in distilled water; and PA-824 was suspended in a cyclodextrin micelle (CM-2) formulation containing 10% hydroxypropyl-β-cyclodextrin and 10% lecithin (18, 22, 23). One volume of drug solution was added to 99 volumes of culture medium, and serial twofold dilutions were carried out; final drug concentrations ranged from 4 to 0.12 μg/ml for RIF and AMK, 1 to 0.12 μg/ml for STR, 0.5 to 0.015 μg/ml for R207910 and MXF, 4 to 0.25 μg/ml for LZD, and 16 to 4 μg/ml for PA-824. Portions (0.1 ml) of the 10^{-1} mg/ml and 10^{-2} mg/ml bacterial suspensions were plated, in duplicate, on both drug-free and drug-containing media. For determination of the MICs of RIF and R207910, which were dissolved in DMF, one of the controls consisted of the same concentration of DMF incorporated into the drug-free medium. The MIC_{50} was defined as the lowest drug concentration that inhibited 50% of the bacterial growth, and the MIC_{90} 90% of the bacterial growth, compared to that on drug-free medium after incubation at 30°C for 60 days.

Quality control was carried out by concomitant determination of the MICs of the same antimicrobial agents against *M. tuberculosis* H37Rv. On 7H11 agar medium, the MICs against H37Rv of RIF, STR, AMK, MXF, R207910, LZD, and PA-824 were 0.25, 2.0, 2.0, 0.25, 0.03, 0.25, and 0.125 μg/ml, respectively (1, 2, 14, 16, 23).

Comparison of the activities against *M. ulcerans* in mice. The left hind footpad of each of the 335 female BALB/c mice, 5 to 6 weeks old, was inoculated subcutaneously with 0.03 ml of a bacterial suspension containing 2.3×10^3 CFU of *M. ulcerans* CU001. Six weeks later, when all mice developed a "lesion index" of 2 or 3 (a lesion index of 2 indicates definite inflammatory swelling, and a lesion

index of 3 indicates severe inflammatory swelling of the inoculated footpad [10]), 10 mice were sacrificed for enumeration of CFU in the inoculated footpads to establish the pretreatment (day 0) value. The remaining 325 mice were randomly allocated among 13 groups (Table 1), including 1 untreated control group of 35 mice, 5 treated groups (treated, respectively, with RIF, STR, or AMK as monotherapy or one of the combinations RIF-STR or RIF-AMK) with 30 mice each, and 7 treated groups (treated, respectively, with MXF, R207910, LZD, or PA-824 as monotherapy or one of the combinations RIF-MXF, RIF-R207910, or RIF-LZD) with 20 mice each. Treatments were begun immediately after randomization. For treatment of mice, RIF, MXF, and LZD were suspended in 0.05% agar-distilled water, STR and AMK were diluted with normal saline, R207910 was dissolved in 20% hydroxypropyl-β-cyclodextrin plus HCl (pH 2), and PA-824 was formulated in CM-2 as described for the experiments in vitro. All antimicrobial agents were given five times weekly by gavage, except for STR and AMK, which were injected subcutaneously. The dosages for each treatment, per kilogram of body weight, were 10 mg RIF, 150 mg STR or AMK, 100 mg MXF, 25 mg R207910, and 100 mg LZD or PA-824, identical to the effective dosages of these antimicrobial agents against *M. tuberculosis* infection of mice.

Severity of the infection and effectiveness of the treatment were assessed by the survival rate and the mean number of CFU per footpad. Mice were sacrificed at regular intervals, with at least 10 mice sacrificed at each time point, as shown in Table 1. To enumerate CFU, the tissues of the inoculated footpad were removed aseptically at sacrifice and homogenized in Hanks' solution in a final volume of 2 ml. In general, each tissue suspension was serially diluted in 10-fold steps, and three appropriate dilutions were plated in triplicate on Löwenstein-Jensen medium and incubated at 30°C for 60 days. For the mice administered RIF-STR or RIF-AMK for 4 or 8 weeks and for those administered RIF, STR, or AMK monotherapy for 8 weeks, CFU were enumerated by plating the entire volume (2 ml) of undiluted tissue suspension from each footpad on 10 tubes of Löwenstein-Jensen medium. For the mice administered MXF, R207910, or LZD as monotherapy or one of the combinations RIF-MXF, RIF-R207910, or RIF-LZD for 8 weeks, a 1:10-diluted suspension was plated in triplicate on Löwenstein-Jensen medium, and 1.5 ml of undiluted tissue suspension was plated on seven tubes of the same medium.

Statistical analysis. Results were analyzed by means of Student's *t* test and Fisher's exact probability calculation. Differences were considered significant at the 95% level of confidence. A regimen was considered bactericidal if the mean number of CFU per footpad in treated mice was significantly lower than the pretreatment value.

RESULTS

MICs of the antimicrobial agents against *M. ulcerans* and *M. tuberculosis*. The range of MICs, the MIC_{50}, the MIC_{90}, and the geometric mean MIC against *M. ulcerans* varied widely among the seven antimicrobial agents (Table 2). The lowest values were observed with R207910; all of these values were several dilutions lower than the corresponding values for the other antimicrobial agents. The next lowest values were seen with MXF. The values of STR were higher than those of MXF but lower than those of RIF, AMK, and LZD; the values of the three latter antimicrobials were virtually identical. The highest values were seen with PA-824. The MICs of RIF, STR, AMK, MXF, R207910, LZD, and PA-824 were 2, 0.25, 1, 0.06, 0.06, 2, and 16 μg/ml, respectively, against the reference strain of *M. ulcerans*, ATCC 19423, and were 2, 0.5, 1, 0.25, 0.12, 1, and >16 μg/ml, respectively, against isolate CU001, which was employed for the mouse experiments.

In six experiments, the MICs against *M. tuberculosis* H37Rv were 0.12 to 0.25 μg/ml for RIF, 0.5 μg/ml for STR, 0.5 to 1 μg/ml for both AMK and MXF, 0.007 to 0.03 μg/ml for R207910, 0.5 μg/ml for LZD, and 0.12 to 0.25 μg/ml for PA-824. All of these values are in agreement with the published values (1, 2, 14, 16, 23).

In vivo activities of various antimicrobial agents against *M. ulcerans* CU001. (i) Survival. As expected (3, 9, 10), 6 of the 25 untreated mice died from *M. ulcerans* infection during the fourth week of treatment (the 10th week after infection), and all 19 surviving mice were seriously ill, with a "lesion index" of 4 (the severe inflammatory swelling had extended from the footpad to the whole limb) (10); therefore, all were sacrificed by the end of the fourth week. Among the 12 treated groups, deaths from *M. ulcerans* infection, as judged by the high "lesion index" of the inoculated footpads, were observed only among the mice treated by PA-824 alone; because 3 of the 20 mice in that group died during the fourth week of treatment and the "lesion index" of the surviving mice was 3.9 ± 0.3, all 17 surviving mice were sacrificed at 4 weeks. All of the mice from the remaining 11 treated groups survived 8 weeks after treatment began, except for one mouse from the group treated with RIF-STR, which died during the first week of treatment because of an accident during gavage. These results demonstrate that all of the regimens, except for PA-824 as monotherapy, prevented death from *M. ulcerans* infection.

TABLE 2. In vitro activities of seven antimicrobial agents against 29 isolates of *M. ulcerans*

Antimicrobial agent	MIC (μg/ml)			
	Range	MIC_{50}	MIC_{90}	Geometric mean
Rifampin	≤0.12–4.0	0.5	2.0	0.56
Streptomycin	≤0.12–1.0	0.25	0.5	0.33
Amikacin	0.25–2.0	0.5	2.0	0.65
Moxifloxacin	0.015–>0.5	0.12	0.5	0.14
R207910	≤0.015–0.12	0.03	0.06	0.03
Linezolid	≤0.25–4.0	0.5	2.0	0.73
PA-824	≤4.0–>16	16	>16	13.1

(ii) Enumeration of CFU in the inoculated footpads. At the beginning of treatment, the mean $\log_{10}$ number of CFU per footpad was 6.35 ± 0.34. As shown in Table 1, the mean number of CFU among untreated control mice had significantly increased to 7.06 ± 0.26 at 2 weeks ($P < 0.01$). Although the value remained at the same level at 4 weeks, six untreated mice had died before the CFU could be enumerated, probably masking a further increase in the CFU between 2 and 4 weeks in the untreated group.

At 2 weeks, CFU were enumerated only among the mice that had been administered RIF, STR, or AMK as monotherapy or RIF-STR or RIF-AMK. All of the values of these groups were significantly smaller than the pretreatment value ($P < 0.05$ or $P < 0.01$), indicating that 2 weeks of treatment by these regimens displayed significant bactericidal activity. The reduction of CFU among the mice administered STR or AMK was significantly greater than that for mice treated by RIF as monotherapy ($P < 0.05$), but there was no significant difference between the results for mice administered the two aminoglycosides ($P > 0.05$). Although the difference between the mean numbers of CFU among the mice administered the combination RIF-STR and those administered STR as monotherapy did not attain statistical significance ($P = 0.059$), the mean number of CFU among the mice administered the combination RIF-AMK was significantly smaller than that among the mice administered AMK as monotherapy ($P < 0.05$).

From 2 to 4 weeks, a significant decline in the mean number of CFU was observed among the mice administered RIF, STR, or AMK as monotherapy or RIF-STR or RIF-AMK ($P <$

0.01). At 4 weeks, only a fraction of the mice in each of the three monotherapy groups were culture positive; neither the proportions of mice with positive culture nor the mean numbers of CFU differed significantly among the groups ($P > 0.05$). All of the mice that had been administered RIF in combination with an aminoglycoside were culture negative. Among the four groups administered the newer antimicrobial agents as monotherapy, the following results were obtained. The mean number of CFU among the mice administered PA-824 did not differ significantly from the pretreatment value ($P > 0.05$). The mean number of CFU among the mice administered MXF or R207910 was significantly smaller than the pretreatment value ($P < 0.05$) but greater than the values among the mice administered RIF, STR, or AMK as monotherapy and sacrificed concomitantly ($P < 0.05$ or $P < 0.01$). The difference between the mean number of CFU among the mice administered LZD as monotherapy and the pretreatment value was marginally significant ($P = 0.06$). Only a fraction of mice administered RIF-MXF, RIF-R207910, or RIF-LZD were culture positive; neither the proportions of mice with positive culture nor the mean numbers of CFU differed significantly among the three groups ($P > 0.05$), nor did they differ significantly from the values among the mice administered RIF either as monotherapy or in combination with an aminoglycoside ($P > 0.05$).

At 8 weeks, various proportions of positive cultures were observed for virtually every monotherapy group except that administered RIF as monotherapy; on the other hand, no positive culture was observed for the mice administered combined therapy, except for a single positive culture observed for mice treated with RIF-R207910. The mean numbers of CFU in mice treated with MXF, R207910, or LZD as monotherapy were significantly smaller than the corresponding values at 4 weeks ($P < 0.05$ or $P < 0.01$), indicating that these monotherapy regimens displayed continued bactericidal activity between 4 and 8 weeks. Although at 4 weeks the mean number of CFU among the mice administered LZD as monotherapy did not differ significantly from the pretreatment value, at 8 weeks the mean number from this group was significantly smaller than the pretreatment value ($P < 0.01$). Although the mean numbers of CFU among the mice administered MXF, R207910, or LZD as monotherapy did not differ significantly from those among the mice administered RIF, STR, or AMK as monotherapy ($P > 0.05$), the proportion of mice with culture-positive footpads among those administered MXF as monotherapy was significantly greater than that among the mice administered RIF, STR, or AMK as monotherapy ($P < 0.05$ or $P < 0.01$), and that among the mice administered LZD as monotherapy was significantly greater than that among the mice administered RIF as monotherapy ($P < 0.05$), demonstrating again that MXF was less bactericidal than RIF, STR, or AMK and that LZD was less bactericidal than RIF.

DISCUSSION

Among the seven antimicrobial agents whose activities had been measured in vitro against 29 clinical isolates of *M. ulcerans*, R207910 demonstrated the lowest MIC, followed by MXF, STR, RIF, AMK, LZD (the MICs of the three last-named agents were similar), and PA-824. The MIC_{90} of each of these antimicrobial agents except PA-824 was below the clinically achievable peak serum level (C_{max}). The MICs of PA-824 were rather high, and its C_{max} data in human are not yet available. That the MICs of the seven compounds against *M. tuberculosis* H37Rv, determined concomitantly with the MICs against *M. ulcerans*, were in agreement with published values (1, 2, 14, 16, 23) indicates that the quality of in vitro cultivation in the study and the potency of antimicrobial agents employed were appropriate.

However, the clinical significance of these in vitro data remains unclear. Moreover, neither the optimal ratio of the C_{max} to the MIC, the ratio of the area under the 24-h plasma concentration curve to the MIC, the free drug area under the concentration-time curve to the MIC, nor the length of time that the drug concentration is above the MIC has been defined for the anti-*M. ulcerans* effect of various classes of compounds. Therefore, more pharmacokinetic and pharmacodynamic studies are needed to assess the usefulness of the in vitro data to predict the in vivo activities of the antimicrobial agents against *M. ulcerans*.

M. ulcerans grows slowly in solid medium. To determine the MICs against *M. ulcerans* on 7H11 agar medium, stability of the antimicrobial agents during the 60 days of incubation at 30°C is a concern. Nevertheless, there appears to be no easy solution other than to follow rigorously the standardized procedures. As shown in Table 2, against 29 clinical isolates of *M. ulcerans*, aside from the MICs of PA-824 (which was inactive against *M. ulcerans* in mice), none of the MIC_{90}s of the remaining six antimicrobial agents (including rifampin, streptomycin, and amikacin) was unreasonably high compared with their MICs against other mycobacteria after a much shorter duration of cultivation. Therefore, we consider the MIC data in Table 2 to be acceptable.

As expected, powerful bactericidal activity against *M. ulcerans* was demonstrated by RIF, STR, and AMK as monotherapy in mice; the activities of the two aminoglycosides did not differ significantly. During the first 2 weeks of treatment, STR and AMK were more bactericidal than RIF, suggesting an earlier onset of killing by the aminoglycosides. However, the advantage of the aminoglycosides was no longer apparent after a longer duration of treatment, similar to the findings that stronger bactericidal and sterilizing activities of STR were observed only during the first 14 days of treatment of pulmonary tuberculosis (16). The bactericidal activities of the combinations RIF-STR and RIF-AMK are very impressive: the mean numbers of CFU in footpads were reduced from pretreatment values by 2 or 3 orders of magnitude (99 or 99.9%) during the first 2 weeks of treatment and by an additional 3 or 4 orders of magnitude (99.9 or 99.99%) between 2 and 4 weeks; all of the footpads of the mice from these two groups were culture negative at 4 and 8 weeks. At 2 weeks, the mean numbers of CFU among the mice administered RIF-STR or RIF-AMK were lower than those among the mice treated by the individual component administered as monotherapy, suggesting a synergistic effect of the combination of RIF with an aminoglycoside, although the synergy was no longer evident at 4 or 8 weeks, at which time the mean numbers of CFU among the mice administered RIF, STR, or AMK monotherapy had declined to a value near the lower limit of detectability.

Of the four newer antimicrobial agents, only PA-824 failed

to prevent mortality of the mice and to reduce the mean number of CFU from the pretreatment value after 4 weeks of monotherapy, indicating that PA-824 is inactive against *M. ulcerans* in mice and confirming the finding that PA-824 possesses a narrow spectrum of activity, limited primarily to the *M. tuberculosis* complex (22). On the other hand, MXF, R207910, and LZD as monotherapy show similar and significant bactericidal activities against *M. ulcerans* in mice, reducing the mean number of CFU by more than 2 orders of magnitude (99%) from the pretreatment value after 4 weeks of treatment and by more than 4 orders of magnitude (99.99%) after 8 weeks of treatment. However, as monotherapy, MXF, R207910, or LZD was less bactericidal than RIF, STR, or AMK.

At 4 or 8 weeks, the mean numbers of CFU per footpad and the proportions of mice with culture-positive footpads among the mice administered any of the combinations RIF-MXF, RIF-R207910, or RIF-LZD did not differ significantly from those among the mice administered either RIF-STR or RIF-AMK, suggesting that all of the former three combinations displayed bactericidal activities against *M. ulcerans* similar to that of the combination of RIF with an aminoglycoside. Because the mean numbers of CFU and the proportion of culture-positive footpads among the mice administered any of the former three combinations also did not differ significantly from those among the mice treated by RIF as monotherapy, one may attribute the promising bactericidal activity of the three combinations mainly to their RIF component. Nonetheless, the bactericidal activity against *M. ulcerans* of MXF, R207910, or LZD would prevent selection of RIF-resistant mutants of *M. ulcerans*, thus justifying their combination with RIF.

The results demonstrate that either MXF, R207910, or LZD may be considered as a companion drug to be administered together with RIF, yielding an effective, orally administered combined regimen for treatment of Buruli ulcer. However, the very high cost and potential toxicity of LZD would limit its application for treatment of Buruli ulcer in the field. Furthermore, R207910 is not yet available for human application. Consequently, at this time, MXF is the only orally administered antimicrobial agent that may be considered as a companion drug with RIF. Additional mouse experiments to define further the activity of the combination RIF-MXF against *M. ulcerans* are warranted. At the same time, because MXF has been found to be well tolerated for the treatment of various clinical conditions (20) and also because several MXF-containing combined regimens for treatment of pulmonary tuberculosis are being evaluated in phase 2 and phase 3 human trials in different parts of the world, a pilot clinical trial to test the efficacy of RIF-MXF for the treatment of Buruli ulcer should be considered.

Long-term (more then several weeks) application of fluoroquinolones in children and adolescents has not been approved because of concerns about potential effects on bone and cartilage growth (4); therefore, the clinical trial of RIF-MXF should be conducted exclusively among adults for the time being. On the other hand, despite class label warnings against use in children, prescriptions for fluoroquinolones to treat infections in children have become increasingly prevalent over the past 10 years (13); in 2004 alone, an estimated 174,000 prescriptions of oral ciprofloxacin were dispensed to the pediatric population in the United States. Moreover, ciprofloxacin was recently approved by the Food and Drug Administration (FDA) for treatment of infections in children (12, 20), and gatifloxacin has also been examined in large-scale studies of pediatric patients (20). Thus, it appears likely that MXF will also be approved for treatment of infections in children, which would allow patients with Buruli ulcer, regardless of their age, to potentially benefit from treatment with MXF.

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